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Effect of α -amylase digestion in fermented Nagara bean grits for gelatinization profile and in vitro starch digestibility

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Abstract. Treatment of α -amylase digestion in wet grits nagara bean after spontaneous fermentation was assessed to determine changes in rehydration ability, gelatinization profile and in vitro starch digestibility of the flour produced. This was important for further processed products that required easy hydration in cold water and high starch digestibility. The research was carried out by hydrolysis of α -amylase with 60 IU enzyme activity as much as 0.1% on wet grits nagara beans from spontaneous fermentation which had been soaked in NaHSO_3 and Ca(OH)_2 for 1 hour. Moreover, α -amylase digestion was carried out at 37°C for 30, 60 and 90 minutes. The results showed that the amylose content of nagara bean flour from the pre-treatment soaking using Ca(OH)_2 was relatively higher than the pre-treatment of NaHSO_3 , and there was a tendency for amylase digestion up to 90 minutes could reduce amylose and starch content. In vitro starch digestibility of flour by amylase digestion of wet grits nagara bean for 60 minutes with pre-treatment soaked in Ca(OH)_2 were 88.79% db, peak viscosity and final viscosity of 2416 cP and 2419 cP respectively.

Keywords: Nagara bean, α -amylase digestion, gelatinization profiles, starch digestibility in vitro

1. Introduction

Nagara bean is one of the adaptive types of cowpea grown in South Kalimantan swamps and has a high carbohydrate and protein content. The protein content ranges from 20-25% and carbohydrate content ranges from 40-60%. Spontaneous fermentation of nagara beans and fermentation by *L. plantarum* can increase protein content if compared to native nagara bean as well as increased in vitro digestibility of starch [1]. The fermentation process is one of the starch modification techniques that affect the solubility, swelling of granule, and starch viscosity [2].

Added-value of the Nagara bean can be increased through product diversification, such as breakfast cereal from the grits of nagara bean and its flour. To reduce acid production during the fermentation process like a slightly sour and aroma, Nagara bean grits were soaked using Ca(OH)_2 and NaHCO_3 . Soaking the wet grits of post-fermented beans is intended to reduce acidity, as well as be able to improve physical characteristics and acceptance of cereal products [3,4], NaHCO_3 is commonly used for snack production through an extrusion process [5].



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Nagara beans that have good rehydration in cold water will improve the performance of breakfast cereal products. Then, to boost the rehydration and its digestibility, α -amylase was used for enzymatic hydrolysis treatment. The enzymatic hydrolysis process that takes place during the fermentation process and the introduction of enzymes into the process are able to change the chemical and physicochemical characteristics and digestibility of flour or starch of beans. Hydrolysis of starch into a lower molecular weight product catalyzed by α -amylase is often to apply and is widely used for food industry applications [6,7].

Increasing food digestibility plays a crucial role to optimize nutrient bioavailability. It is closely related to the applied technology that carried out using size reduction, soaking, boiling, and fermentation processes. Flake cereal can be developed through flaking on the nagara bean grits or its flour. Hydrolysis grits by α -amylase were purposed to produce the grits easy to hydrate, to gelatinize and to digest.

Starch granules are slightly resistant by water penetration and hydrolytic enzymes because of hydrogen bonds within a molecule and other molecules. The α -amylase is categorized as an endoamylase found in many microorganisms [7]. According to [8-10] endoamylase catalyzes hydrolysis randomly on the inside of starch molecules, this results the formation of linear and branched oligosaccharides of various chain lengths. Endoamylase cuts 1-4 glycosidic bonds which are on the (endo-) amylose or amylopectin side.

The research was aimed to determine the changes in the the gelatinization profile of nagara bean and in vitro digestibility of starch in the grits of wet nagara beans hydrolyzed (digestion) by α -amylase.

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2. Materials and method

2.1. Materials and instruments

Nagara beans were obtained from the Nagara Hulu Sungai Selatan area as a part of South Kalimantan. Pepsin from gastric mucosa 250 units/mg, α -amylase from porcine pancreas Type VI-B 10 units/mg, amyloglucosidase from *Aspergillus niger* 30-60 units/mg, amylose standard, 3,5-Dinitrosalicylic acid, Folin-Ciocalteu reagent, NaOH, Na₂CO₃, Na K-Tartrat, CuSO₄·5H₂O, ethanol, trichloroacetic acid, potassium iodide, Iodine were used. The tools such as the Waterbath shaker (Memmert), oven (Memmert), Spectrophotometer (Mapada), centrifuge, vortex and glassware were operated for chemical analysis.

2.2. Fermentation process

Fermentation was done on the nagara bean grits using the ratio between bean and water was 1:4 for 48-hours fermentation periods. The fermented beans were washed and peeled and then drained.

2.3. The treatment process

Nagara bean grits produced from spontaneous fermentation for 48 hours were neutralized by soaking using Ca(OH)₂ and NaHCO₃ up to 200 ppm for 1 hour. Nagara beans grits were washed and then digested by enzyme at a ratio of 1:2 of bean and water added α -amylase concentration of 0.1% (v/w) at 37°C for 30, 60 and 90 minutes respectively, then drained and dried for 48 hours at 60°C and grinded up to 80 mesh.

2.4. The parameter of analysis

The nagara bean flour was analyzed for several parameters such as amylose contents [11], reduced sugar content (DNS method), starch contents (Luff schroll method), resistant starch, digested starch [12] and in vitro starch digestibility [46]. Gelatinization profile by using Rapid Visco Analyzer Model RVA-S4 with the Thermocline for Windows (TWC) program.

2.5. Starch digestibility in vitro [46]

The flour suspension (1%) in test tube was heated in a water bath at 90°C for 30 minutes then cooled. The amount of 2 mL flour suspension was added distillate water of 3 mL and 5 mL of Na-phosphate

7 buffer 0.1 M pH 7 then was incubated in a water bath at 37°C for 15 minutes. Moreover, 5 mL of α -amylase solution was added and then incubated at 37°C for 30 minutes. Take 1 mL of the solution, then 12 added with 2 mL of dinitrosalicylic reagent and heated 100°C for 10 minutes. After cooling, the solution was diluted by 10 mL of distilled water. The absorbance of the solution was measured by using a spectrophotometer at a wavelength of 520 nm. Maltose content was measured using the standard pure maltose curves, starch digestibility is calculated as a percentage relative of maltose content sample to its pure starch.

2.6. Resistant starch and digested starch [27, 2]

Flour samples of 100 mg were 1 dissolved in 10 mL HCl-KCl buffer at pH 1.5 then vortex 1 minute, 30 added 100 μ L pepsin solution (0.1 mL-10 mg pepsin/mL 24 HCl-KCl, and then incubated 60 minutes at a temperature of 40°C. The tube was added a solution of α -amylase 1 mL (40 mg/mL tris maleate solution) and incubat 32 at a temperature of 37°C for 16 hours at pH 6.9 to hydrolyze the digested starch, then the sample was centrifuged for 15 minutes of 4500 rpm 6 the supernatant was removed, then the residue was added 2M KOH of 6 mL and an 80 μ L (140 U/mL) of am 6 glucosidase enzyme solution and incubated at 60°C for 45 minutes at pH 4.75. The samples were centrifuged at 4500 rpm 28 15 minutes. The supernatant was measured glucose levels using the DNS method. Digested starch was calculated based on the total amount of starch reduced by the amount of resistant starch.

2.7. Data analysis [10]

The data were analyzed by analysis of variance (ANOVA) at an error rate of 5%, and then proceed using a Duncan Multiple Rate Test (DMRT) test if a real effect of treatment was available.

3. Result and discussion

3.1. Amylose content

Starch was allocated to two fractions such as amylose and amylopectin containing 20-30% of amylose and 70-80% of amylopectin [13], with an example of Barley containing 29.8% of amylose [14] and wheat of 21.5- 26.6% [15].

The results showed that α -amylase digestion in some treatments had an effect on the amylose content. The α -amylase digestion processes produced 18.49-25.38% db of amylose Nagara bean flour. Enzymatic modification 9 shattered weak starch granules and caused pores in the surface area of starch. This would reduce the ability to bind water and to decrease viscosity. Amylose was broken into simple molecules reducing the amount of amylose starch and an excellent swelling volume [22].

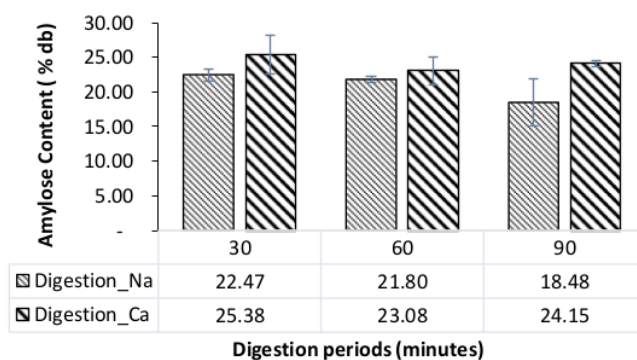


Figure 1. The intercorrelation among enzymatic digestion periods and the amylose content of Nagara bean flour.

Figure 1 shows the amount of amylose in Nagara bean flour. The digestion period of α -amylase was 90 minutes tending to a decrease of amylose content. This also correlated with an increase of amylopectin content which produces high viscosity at low temperature [14].

Wet Nagara bean grits with a neutralizing pre-treatment using $\text{Ca}(\text{OH})_2$ tended to have a high amount of amylose. Gomez *et al* (1989) stated the alkali process of corn and wheat damaged starch granules in endosperm causing to lose its crystallinity giving an effect on the opening of amylose structure [16]. Similarly, Berrios *et al* (2004) stated that the addition of NaHCO_3 expanded matrix starch and protein bonds became softer and were elongated with an increase of NaHCO_3 concentration [17]. An increase in amylose was in line with gelatinization temperature; however, the paste viscosity was low [18].

According to [19] and [20], the Ca^{2+} , Mg^{2+} , Mn^{2+} and Na^+ ions at 5mM concentration increased amylase activity. Calcium could protect the amylase molecule from protein hydrolysis. It prevented the configuration of amylase protein remaining biological activity and stabilizing the secunder and tertier structures of the enzyme [21].

3.2. Reducing sugar content

The α -amylase (EC 3.2.1.1) was an enzyme catalyzing internal hydrolysis processes of α -1,4 glycosidic bonds of starch into products with low molecules such as glucose, maltose and maltotriose [23-26]. Figure 2 described that the reduced sugar content of Nagara bean flour using α -amylase digestion treatment was 2.17-2.27 mg/mL showing a decrease with the digestion period up to 90 minutes.

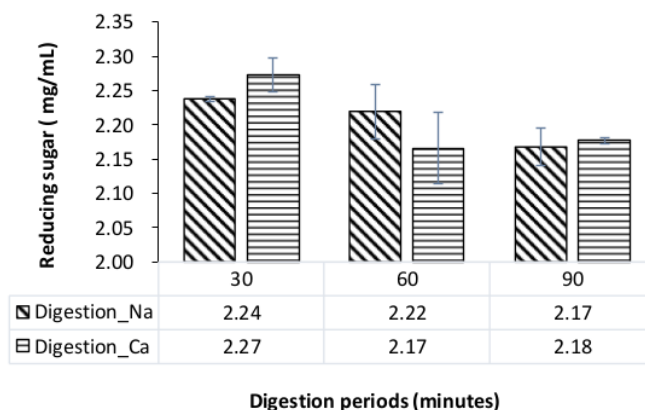


Figure 2. Intercorrelation between enzymatic digestion periods and sugar reduction content of Nagara bean flour.

The content of sugar reduction showed a low value after α -amylase hydrolysis because, in hydrolysis processes of raw Nagara bean grits, α -amylase enzyme attacked Nagara bean matrix more slowly; therefore, resulting in a low cutting glycosidic bond. In addition, the process of gelatinization and non-gelatinization preparation affected the intensity of the α -amylase hydrolysis process. Konsula and Liakopoulou-Kyriakides (2004) investigated the gelatinized potato starch and rice starch were more easily degraded by the enzyme α -amylase than in the native form [27].

The hydrolysis process of native granules using commercial enzymes varied depending on the material type, porosity and features of the surface area [28,29]. Nagara bean contained raffinose oligosaccharides, and the structure of cellulose and hemicellulose which allowing α -amylase hydrolysis were not sufficient to shatter cellulose into hexose sugar. It was supported by [30] carbohydrates in sorghum were resistant to enzymatic hydrolysis due to cellulose crystallinity, surface accessibility and the ratio between hemicellulose and cellulose.

According to [29] each native starch varied porosity and the changes of starch porosity were caused by mechanic treatments such as size reduction, grinding and in situ amylase processes. Native starch granules were inert to chemical reactions causing the activity of pre-treatment which was an example from enzyme hydrolyze that could increase the porosity of starch granules which chemically affect the chemical reactivity of starch. Enzymes hydrolysis processes were affected by the temperature of 37°C; however, Konsula and Liakopoulou-Kyriakides (2004) showed an increase of temperature resulting in a high reduction of sugar [27].

3.3. Starch content

The Nagara bean contains a large amount of protein and carbohydrates of 50-60%. Starch was part of carbohydrate polymers as a component that plays a role to produce textures and to be used in industrial applications such as thickener, stabilizer, gelling agent and water retention agent [31]. The starch content of Nagara bean flour from the amylase digestion process ranged from 50.88 to 53.33%db. The result pointed to have no significant effect between α -amylase digestion and the starch content of Nagara bean flour as shown at figure 3.

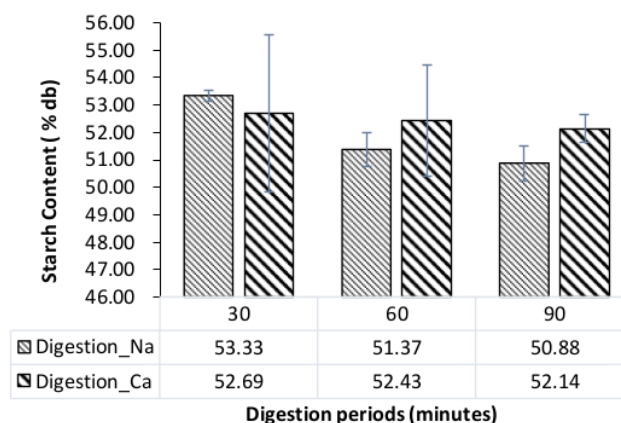


Figure 3. Intercorrelation between enzymatic digestion periods and starch content of Nagara bean flour.

There was a tendency to slightly decrease the starch content when α -amylase digestion increased up to 90 minutes. However, a sharp decline in starch contents happened during NaHCO_3 pre-treatment compared to Ca(OH)_2 . It is assumed that the ability of Ca^{2+} bonded in matrix gave an effect of cross bonds performing a high bond of intra and intermolecules causing a low degraded starch. However, the presence of NaHCO_3 tended to produce pores in the surface area and to reduce molecule bond strength. Likewise, another study stated Ca^{2+} had triggered off α -amylase activity [19].

3.4. Resistant starch

Some legumes are sources of carbohydrate-containing dietary fiber [32] and a slow-digestible starch and resistant starches [47]. Resistant starch was defined as starch and starch degradation products that cannot be absorbed in the small intestine in healthy individuals [33]. Type RS 1 was starch which was physically inaccessible, meanwhile, type RS 2 was natural starch granulated which was not gelatinized and difficult to be degraded by amylolytic enzymes because the structure was compact and anhydrous. Type RS 3, was retrograded starch, and RS 4 was formed from chemical modification [33, 34]. Resistant starch was measured by the enzymatic method, data of resistant starch content were presented in figure 4.

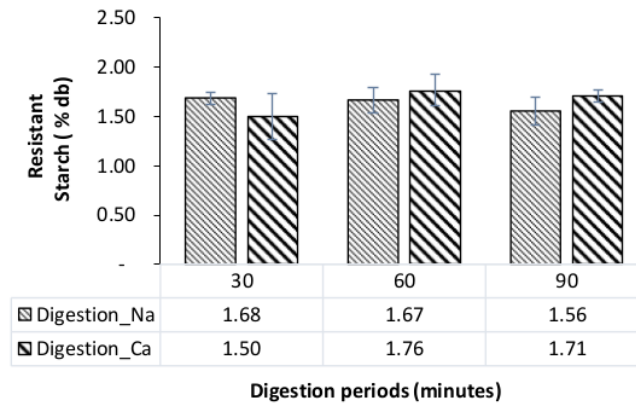


Figure 4. Intercorrelation between enzymatic digestion periods and resistant starch content of Nagara bean flour.

Figure 4 showed no differences using $\text{Ca}(\text{OH})_2$ and NaHCO_3 in the flour of Nagara bean grits using α -amylase digestion. The resistant starch content in Nagara bean flour was $3.07 \pm 0.07\%$ db, with the hydrolysis process of α -amylase tend to reduce the resistant starch. While some studies showed that resistant starch in cereals changed due to the treatment of moist-l²⁶ processes, autoclaves or autoclave-cooling cycles, conventional cooking processes such as high-pressure steaming, microwave, autoclaving, soaking, and boiling which reduced the amount of resistant starch [34-36].

3.5. Digested starch

Digested starch²² calculated based on a reduction in the total amount of starch with the amount of resistant starch, digested starch including rapidly digestible starch (RDS) and slowly digestible starch (SDS). Figure 5 showed that the total digested starch in the treatment using Na_2CO_3 and $\text{Ca}(\text{OH})_2$ tends to be relatively stable at around 51%. The treatment of α -amylase digestion did not significantly affect the change in the amount of resistant starch so that changes in digested starch were also relatively not significantly different between treatments.

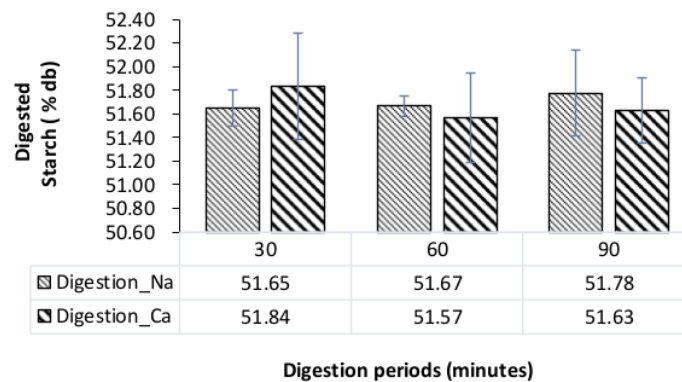


Figure 5. Intercorrelation between enzymatic digestion periods and digested starch content of Nagara bean flour.

Research by [37] mentioned the enzymatic hydrolysis process using pullulanase in rice starch [14] decreased the rapidly digestible starch (RDS) fraction and increased slowly digestible starch (SDS) and resistant starch (RS). While in this study the fermentation process and amylase digestion reduced resistant starch, so that digested starch tended to be higher than in untreated beans. According to the Nilegaonkar [38], the autoclave process could decline the value of starch digestion index (SDI) in legumes. The autoclave method decreases starch digestibility and rapidly digested starch. The method could convert resistant starch (RS) and rapidly (RDS) digested starch into slowly digested starch (SDS). It was assumed that the pressurized steam preconditioning caused a reduced RDS, with the long period of intensively decreased steaming. Vatanasuchart *et al* (2009) explained that processed snack products from rice have a resistant starch of 2.9%db, there is an increase in starch resistant from the raw material (RS type II) [39]. This indicates the presence of resistant starch produced from retrogradation (RS type III) due to the given process [40].

3.6. Gelatinization profiles

Some changes occur during the heating of the starch-water system including swelling power, increased viscosity, transparency and solubility and loss of anisotropy (birefringence), the change was known as gelatinization. Gelatinization causes the changes in the chemical and physical properties of starch granules because of the intra-rearrangement and intermolecule of hydrogen bonds between water and starch molecules, resulting in molecular damage in starch granules.

The high initial gelatinization temperature indicated the granule was resistant to swell. Paste temperature is one of the characteristics of the paste indicating the minimum temperature needed for cooking, the energy costs needed and the stability of other components. Peak viscosity also showed the nature of the water-binding capacity of the starch. Final viscosity is used to define starch quality and shows stability in actual use, it can also be used to demonstrate the ability to form pastes or gels after cooling. The gelatinization profile of nagara bean flour is presented in table 1 and figure 6.

Table 1. The gelatinization profiles of Nagara bean flour by α -amylase digestion.

Treatments		Gelatinization profiles						
Neutralizing agent	Amylase digestion periods (minutes)	Peak visc (cP)	Trough visc (cP)	Break down visc (cP)	Final Visc (cP)	Seat back visc (cP)	Peak Time (min)	Pasting temp ($^{\circ}$ C)
GritsNa	30	2333	1275	1058	2293	1018	8.13	80.15
	60	2365	1227	1138	2298	1071	8.00	80.05
	90	2480	1206	2417	2417	1143	8.07	79.70
GritsCa	30	2369	1183	1186	2260	1077	8.13	80.10
	60	2416	1249	1167	2419	1170	8.00	79.70
	90	2391	1243	1148	1222	1079	8.13	80.10
Control		2093	1074	1019	1617	543	7.53	78.10

Table 1 shows the enzymatic digestion treatment provided a significant gelatinization profile where the highest peak viscosity was obtained in the 90 minutes enzymatic digestion of grits by NaHCO_3 treatment of 2480 cP with 79.7 $^{\circ}$ C paste temperature while $\text{Ca}(\text{OH})_2$ neutralized treatment resulted in peak viscosity of 2416 cP with 60 minutes digestion periods. There was a tendency for increasing digestion periods up to 90 minutes to increase peak viscosity, seatback, and final viscosity. Digestion for 60 minutes, the grits with the pre-treatment by $\text{Ca}(\text{OH})_2$ [13] tend to have a higher peak viscosity than the pre-treatment using NaHCO_3 . According to [41], divalent cations (Ca^{2+}) produce greater viscosity than monovalent (Na^+) cations due to inter or intramolecular cross bonds in polymer chains. Research by Chen *et al* (2014) [31] showed that the addition of NaCl to the flaxseed and potato complex starch can also increase paste temperature, peak viscosity, final viscosity and breakdown value [42].

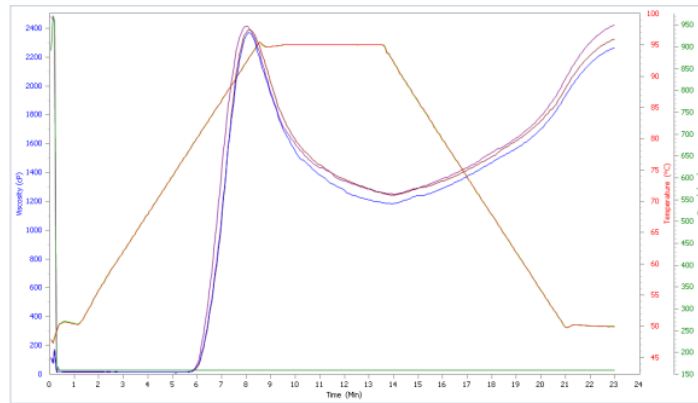


Figure 6. Gelatinization profiles of Nagara bean flour using amylase digestion with Ca(OH)₂ neutralizing pre-treatment.

According to Feng *et al* (2016) the addition of NaCl slightly increases peak viscosity, final and setback viscosity, while the presence of CaCl₂ reduces peak, final and breakdown viscosity [43]. The high peak viscosity indicates a high starch content, which closely related to water binding capacity in starch [44]. Nagara bean flour with high peak viscosity is suitable for products that require high and elastic gel strength.

3.7. Starch digestibility in vitro

Starch hydrolysis showed the function of starch in plants as well as food sources [45]. In vitro starch digestibility illustrates the ease of digestive enzymes, especially amylase enzymes, to degrade and hydrolyze starch into short chains so that it is easier to be utilized and absorbed in the body. In vitro starch digestion in nagara bean flour from amylase digestion ranged from 76.70-88.79% db, this value was higher than starch digestibility in vitro of steamed nagara bean grits [1]. It is suspected that further amylase digestion process increases the porous structure of the starch so that it is more easily digested, the starch matrix is more accessible to the amylolytic enzyme, while the steaming process can cause the pre gelatinization process so that it can increase the crystalline structure which is more difficult to attack the amylase enzyme.

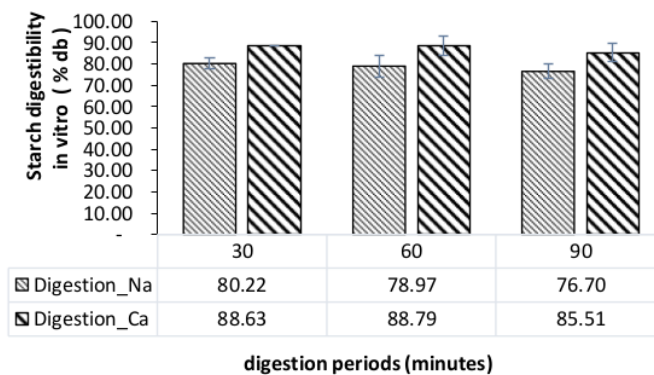


Figure 7. Intercorrelation between enzymatic digestion periods and starch digestibility in vitro of Nagara bean flour.

4. Conclusion

The hydrolyzed wet Nagara bean grits by α -amylase had a higher flour gelatinization profiles such as peak viscosity, setback viscosity and final viscosity rather than the unhydrolyzed Nagara bean flour. Wet grits of Nagara bean were carried out by neutralizing pre-treatment using $\text{Ca}(\text{OH})_2$ and digested by α -amylase for 60 minutes had peak viscosity was higher than they were neutralized by NaHCO_3 . Likewise, in vitro starch digestibility of flour was neutralized by $\text{Ca}(\text{OH})_2$ which was relatively higher than neutralized by NaHCO_3 .

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